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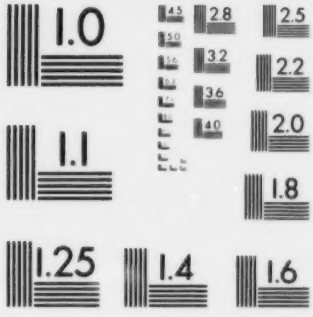

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MICROBIAL CONTROL OF ACID LEACHING FROM MINE TAILINGS

(Contract Number 1ST85-00262)

Final Report

December, 1987

Submitted to Dr. D. Gould, CANMET,

by D.J. Kushner, T. Kauri and D.A. Brown, Department of Biology,

University of Ottawa

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28 pp

MICROMEDIA

ABSTRACT

This report is a continuation of the one submitted in 1986, and represents an additional half-year's work. Previously, methods were developed for measuring *in situ* production of methane. These methods have now been used extensively to measure one field site; in addition, a good correlation was shown to exist between methane production in the field and by samples from the same site and horizon incubated in the laboratory.

We had previously shown data for the bacterial population of one peat sample. This work has now been extended to several well-characterized peat samples, including some with quite different chemical and physical properties taken from approximately the same geographical location. A study was also made of the fungal species found in these samples, and some estimates were made of the protozoa as well.

Overall, our methods should permit us and other to predict and measure the long-term ability of different kinds of peat bogs to maintain an anaerobic cover over mine tailings.

SOMMAIRE

Ce rapport, la suite de celui de 1986, est le fruit d'un autre six mois de travail. Auparavant, la quantité de méthane produite in situ était mesurée au moyen de méthodes qui avaient été conçues à cette fin. Celles-ci ont été largement utilisées pour déterminer la quantité de méthane d'un emplacement expérimental. De plus, il a été possible d'établir une corrélation étroite entre la production de méthane in situ et dans les échantillons qui ont été prélevés du même emplacement et horizon et incubés en laboratoire.

Nous avons déja présenté des données relativement à la communauté bactérienne d'un échantillon de tourbe. Nous avons maintenant étendu la portée de ce travail à plusieurs échantillons dont les caractéristiques ont été bien déterminées, y compris certains dont les propriétes chimiques et physiques différent énormément et qui ont été prélevés approximativement dans la même région. Les moisissures retrouvées dans ces échantillons ont également fait l'objet d'une étude. De plus, certaines estimations ont été faites des protozoaires.

En somme, grâce à ces méthodes, il devrait être possible de prévoir et mesurer à long terme lacapacité des différents genres de marécages de tourbe de maintenir une couche anaérobique au-dessus des déchets de mine.

RESULTS OBTAINED

I. METHANE FORMATION

Field sampling of methane

The sampling tubes, as described in the previous report (Brown et al, 1986; Dinel et al, 1987) were made up in stainless steel over the winter and proved to be effective in the field. A field methane monitor was tested, but was not found to be useable in its present form, since it was not possible to obtain a satisfactory flow of gas from below the surface. Therefore, gas bottles, manufactured by Scienceware, of Bel-Art Products (see previous report) were used to collect samples, and methane determinations of the collected gas were made later on a Hewlett-Packard gas chromatograph.

An array of stations was set up in Mer Bleue, near Ottawa (Fig. 1) for field measurements of methane. Twenty-five stations were sited in a square at 6 m intervals. During the end of September-beginning of October, 1987, methane was collected at three depths, 60 cm, 90 cm and 120 cm. Five bottles were collected at 15 min intervals for each level at each site; nearly 400 readings were taken in all.

The amounts of methane extracted are shown in Fig. 2. In the main, the major portion of the gas was collected in the first 30 min. At the times when no methane is shown, the tube was faulty and did not maintain its vacuum. However, except where this was

the final tube for the hole, the methane was collected in the succeeding tube. The total umoles of methane obtained from each hole is shown, in contour lines, in Fig. 3. The total amount from the three depths at each site is also shown; this amount has been divided by three to normalize it so that it may be compared to that found at each depth.

The amount of water extracted has been shown in similar contour lines in Fig. 4. The maximum amount of water that can be sucked up by each tube, with the suction we are able to apply, is 200 ml, so that the total possible amount of water is 1000 ml. However, many tubes did not contain water, and there were only two sites where more than 600 ml of water were collected.

These results show that there is no correlation between the amount of methane and of water extracted. In fact, possibly due to the exceptionally dry summer this year, in many cases we were not able to extract sufficient water or gas to bring the bottle to atmospheric pressure. Unfortunately, the implications of this -- that is, that any methane present might not be free to flow into the tubes because of back-suction -- were not realized soon enough for the excess suction to be measured. The normal practice was to equilibrate the gas in the bottle with the atmosphere by allowing the bottle to fill with air. However, once the significance of the suction was realized, the tubes were equilibrated with water, which could be measured. It appears that Mer Bleue, unlike the Gatineau bog sampled last year, is very impermeable; several samplings are probably necessary to show the true amount of methane found in a given site. Future work should include

techniques for flushing out methane from impermeable sites, so that accurate measurements can be made with a single sample.

The amount of methane extracted in individual samples varied from 14 umoles to 3189 umoles at the 60 cm depth, from 22 umoles to 8262 umoles at the 90 cm depth, and from 40 umoles to 4884 umoles at the 120 cm depth. The total for each hole varied from 1137 umoles to 10465 umoles. Some of the the lower values are probably artifically low, due to malfunctioning of the sampling tube. Although the amount of methane varies considerably, the amount of extractable methane in the bog seems to be relatively high. Four test holes have been done in the Sudbury area, and the methane extracted from these holes ranged from 9 umoles to 508 umoles. This is very encouraging, as the bogs there are much shallower (ca. 60 cm deep) than the 2.5 m depth of Mer Bleue.

We still do not know if the variability is due to difficulty of extracting the methane of whether, because of the heterogeneous nature of the peat substrate, the methane is differentially produced. However, the amount of methane appears to be sufficient to provide a good anaerobic environment.

Laboratory incubations

Peat samples were taken from four of the sites, at the same depths as those in which the methane was measured. The samples were incubated in the laboratory at 25 C under anaerobic conditions. The results were calculated in nmoles of methane produced per gram dry peat per hour. When the results were plotted (Fig. 5) against the amount of methane extracted from the same

sites, a surprisingly good correlation of r= 0.8145 was obtained. This suggests that the amount of methane extracted from the peat is dependant on the activity of the organisms in that portion of the peat. In addition, laboratory measurements of production of methane by peat samples should give a good indication of methane production to be expected in the field.

MEASUREMENTS OF SULFUR COMPOUNDS

A hydrogen sulfide detector, HS267, manufactured by Industrial Scientific Corporation, was used to monitor gas from the methane sampling tubes taken in the Mer Bleue, but no H_2S was detected. The turbidity method (EPA 1979) was used to detect sulfates in water from this bog, but again none was detected. Thus, sulfate reduction does not seem to play an important role in this bog.

ROLE OF CELLULOSE IN RESPIRATION OF PEAT

In our previous report, we noted the correlation between cellulose content of different peat samples and the respiration of these samples. "Cellulose" was previously measured mainly as total sugars obtained after acid hydrolysis. The method used did not, however, distinguish between glucose and other sugars. Gas chromatograph measurements of peat hydrolysates have now shown that glucose is by far the major sugar released by acid

hydrolysis. Considering the insoluble nature of the polymer before hydrolysis, it is highly likely that the glucose is derived from cellulose and that this compound is the main one responsible for increased respiration.

MICROBIOLOGICAL STUDIES

In our previous report, we described the bacterial population of one peat sample (a humisol from Noranda) at different depths. The techniques used have now been extended to other samples, with different chemical and physical properties.

Aerobic bacteria

The methods used for determining bacterial and spore counts, as well as for determining the numbers of cellulolytic bacteria were as previously described (Brown et al, 1986). Samples were collected from the Noranda area at two different depths (10 cm and 45 cm). The three types of muskeg bog described previously (humisol, mesisol, and fibrisol) were located around the same area, but have different compositions and structure. Viable counts and spore counts of the three bogs are shown in Fig. 6. In the surface layer, the highest bacterial counts were found in humisol, followed by mesisol, then fibrisol. In the deeper level, the counts were considerably lower. Again, humisol had the highest counts, follwed by mesisol and fibrisol.

Spore counts were three times as high in mesisol as in humisol and fibrisol in the surface layers, while in the deeper layers mesisol had the lowest spore counts and humisol the highest.

Aerobic cellulolytic bacteria in the three bogs at two different levels are shown in Fig. 7. The highest numbers of

cellulolytic bacteria were found in humisol and the lowest in fibrisol. In the deeper layers, mesisol and, especially, fibrisol had very low numbers of cellulolytic bacteria. These results confirm that there is a clear difference in the bacterial populations of the different types of muskeg bogs.

There is an abundant and diverse population of metabolically active aerobic bacteria in muskeg bogs. The numbers diminished with increasing bog depth, but still showed quite high levels of both total viable bacteria and cellulolytic bacteria, especially in the humisol bog. Spore-forming bacteria made up a small part of the total population, except in the mesisol surface layers.

Fungi

Soil samples were treated as described in our previous report (Brown et al, 1986). Dilutions were plated on malt extract agar, with addition of 0.12% yeast extract, as well as on Sabourard's agar. Streptomycin (40 ppm) and penicillin (15 ppm) were added to plates to prevent bacterial growth. Plates were incubated at 25 C. Fungi were identified at the genus level by morphological characteristics.

Peat soil samples were collected from the Noranda area (humisol) at two depths, 10 cm and 45 cm. One sample (40 cm depth) from the Falconbridge area was of a reclaimed wetland which is returning to marsh. Another sample was taken from the surface of a Gatineau bog. The Gatineau sample was most acid, with a pH of 3.8, whereas the Noranda and Falconbridge samples had pH's of 5 and 6 respectively.

Fig. 8 shows the number of fungi in different samples, as measured by colony-forming units. The numbers in the surface samples are considerably higher than in the deeper layers.

Penicillium, Trichoderma, Verticillium and Mucor were found in all the samples, except that the Gatineau bog contained no Mucor and no Verticillium was found in the sample from Falconbridge. Not all the fungi in all samples were readily identifiable.

The fungi from the surface layers seemed to display more vigorous growth than the others. In the surface layers of the Noranda and Gatineau samples, Penicillium, Trichoderma and Verticillium dominated the plates. Competition between the different fungi presumably limited the number of genera present. The species from the two bogs seemed different: the plates containing the Gatineau bog samples were dark coloured, while in those containing the Noranda samples the mycelium was extensive and cottony.

In the deeper bog layers, a greater variety of fungi was found. The Falconbridge samples contained, in addition to the species already listed, colonies of Cephalosporium, as well as Botrytis, which was also found in the deeper layers of the Noranda bog. The latter was dominated by Chrysosporium and Cephalosporium, but only isolated colonies of Penicillium, Trichoderma, Verticillium and Mucor were found. Sporotrichum was found only in the Noranda bog sample. The diversity shown in the deeper layers of the Noranda sample could be due to the low rate of growth of the funi present: the lack of any overgrowing species gave a

better opportunity for many different types to develop on the plate. As fungi are primarily aerobic organisms, they probably make no significant contribution to turnover below the peatland surface.

Rossi-Cholodny technique

For direct observation of microorganisms present in peat, pairs of microscopic slides, pressed together, were buried in Gatineau and Mer Bleue peat. They were removed after two months and examined microscopically, using both wet mounts and stained preparations.

Relatively few microcolonies could be observed. Most of the microorganisms present were bacteria, single or in pairs. This suggests that the cells did not divide very often. Many spores were seen. There were also filamentous bacteria and blue-green algae. A few eucaryotic algae, yeast colonies and short lengths of fungal mycelia were also found. Except for a few cysts and flagellates, no protozoa were found. A further search for these was made on samples of pore water and debris from peat.

Protozoa

Samples of pore water and debris from peat were collected from aerobic layers. Phase-contrast microscopic examination was carried out within 24 hour of sampling.

Quantitative measurements of the number of protozoa were not made. It was noted, depending on the sample, that the "numerous" organisms appeared in approximately every fifth field in the

microscope (400 x magnification) and the less frequent ones appeared only in about every twentieth field.

Encysted stages were most numerous in the samples, along with thecae of shelled amoebae, which prediminatly belonged to the arenaceous type. Flagellates and ciliates were found less frequently in these samples. Of the former, euglenoid phytoflagellates were seen, while the ciliates were represented by smaller holotrich forms.

The samples were examined only once. It was thought that a continuous examination over a longer period of time and/or under varied culture conditions would likely have revealed other forms as well. This, however, would require a separate investigation.

OVERALL CONCLUSIONS

The work for the past year and a half has enabled us to characterize a large number of peat bogs chemically and physically and to characterize a selected number of these bogs microbiologically. Methods of measurement of methane formation were developed and tested, both in the laboratory and in the field. A correlation between cellulose content and soil respiration in the different bogs was established. This provides a relatively simple way of predicting which bogs will have the highest potential for degradation and, hence, might maintain the most anaerobic conditions.

Overall, our studies should help us, or other workers, to predict the kind of bogs that might be best suited for maintaining

long-term anaerobic conditions. This work will also allow the testing of the maintenance of anaerobic conditions of bogs which have been placed on top of mine tailings. Such tests, in turn, should give an estimate of the effectiveness of this cover in preventing acid leaching.

REFERENCES

A. Brown, D.J. Kushner and T. Kauri. 1986. Microbial control of acid leaching from mine tailings. report to CANMET, 38 pp, with appendices.

H. Dinel, S.P. Mathur, A. Brown and M. Levesque. 1987. A field study of the effect of depth on methane in peatland waters - equipment and preliminary results. J. Ecol. in press.

EPA (U.S. Environmental Protection Agency) 1979. Methods for chemical analysis of water and wastes. Report No. EPA-600/4-79-020.

FIGURE LEGENDS

Figure 1. Map of Mer Bleue showing the methane sampling site.

Figure 2. Bar graphs of the amount of methane extracted over time at 60cm (short), 90cm (medium) and 120cm (long) depth, for each sampling site.

Figure 3. Contours in mmoles of methane extracted from the array of 25 sampling sites at 60cm, 90cm and 120cm depth. Total amount of methane for each site was normalized so it could be compared to that found at each depth.

Figure 4. Countours in cl of water extracted from the array of 25 sampling sites at 60cm, 90cm and 120cm depth. Total amount of water for each site was normalized so it could be compared to that found at each depth.

Figure 5. Correlation of methane extracted in the field with the methane produced from samples incubated in the laboratory from the same site and depth.

Figure 6-8: The legends are placed below each figure.

MER BLEUE

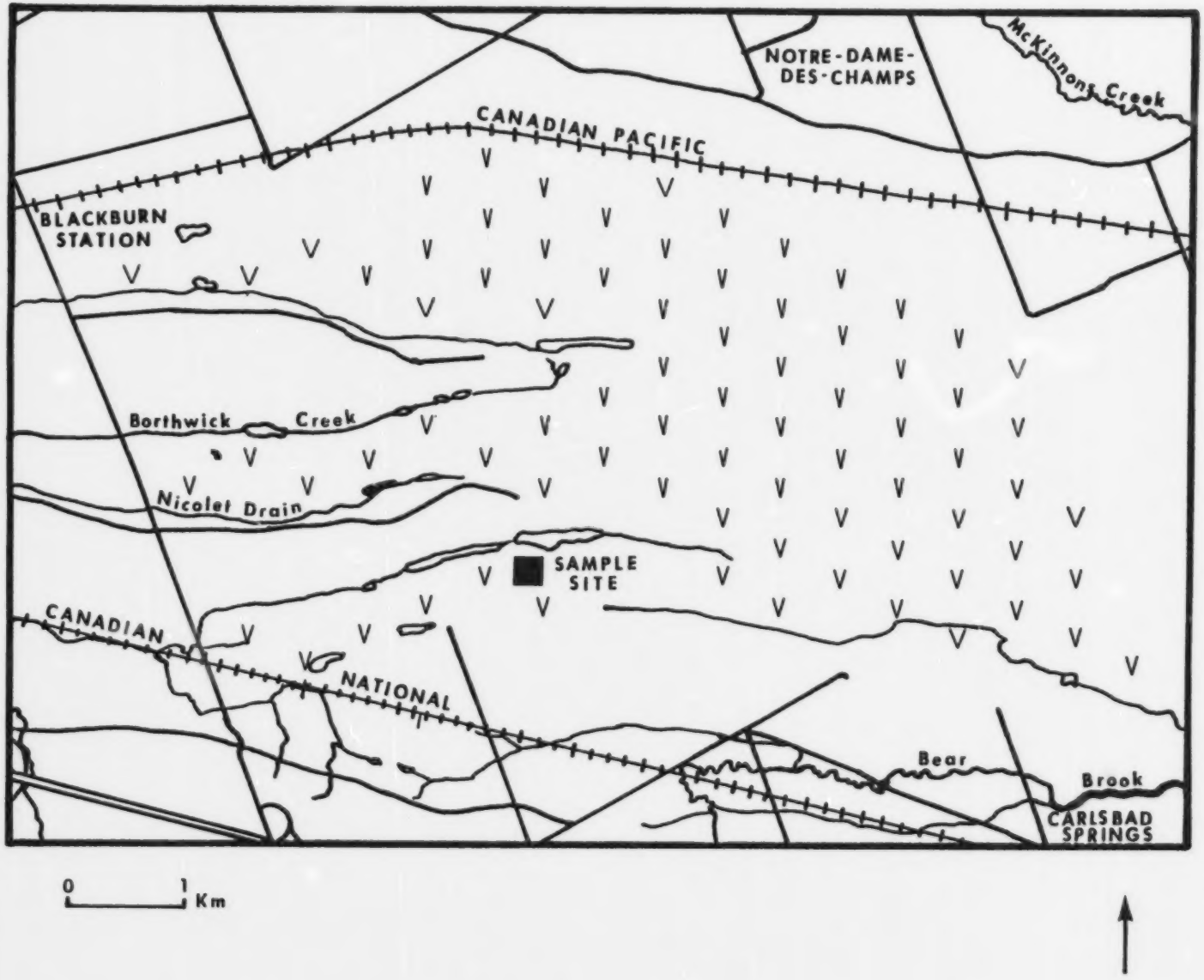


Figure 1

Figure 2

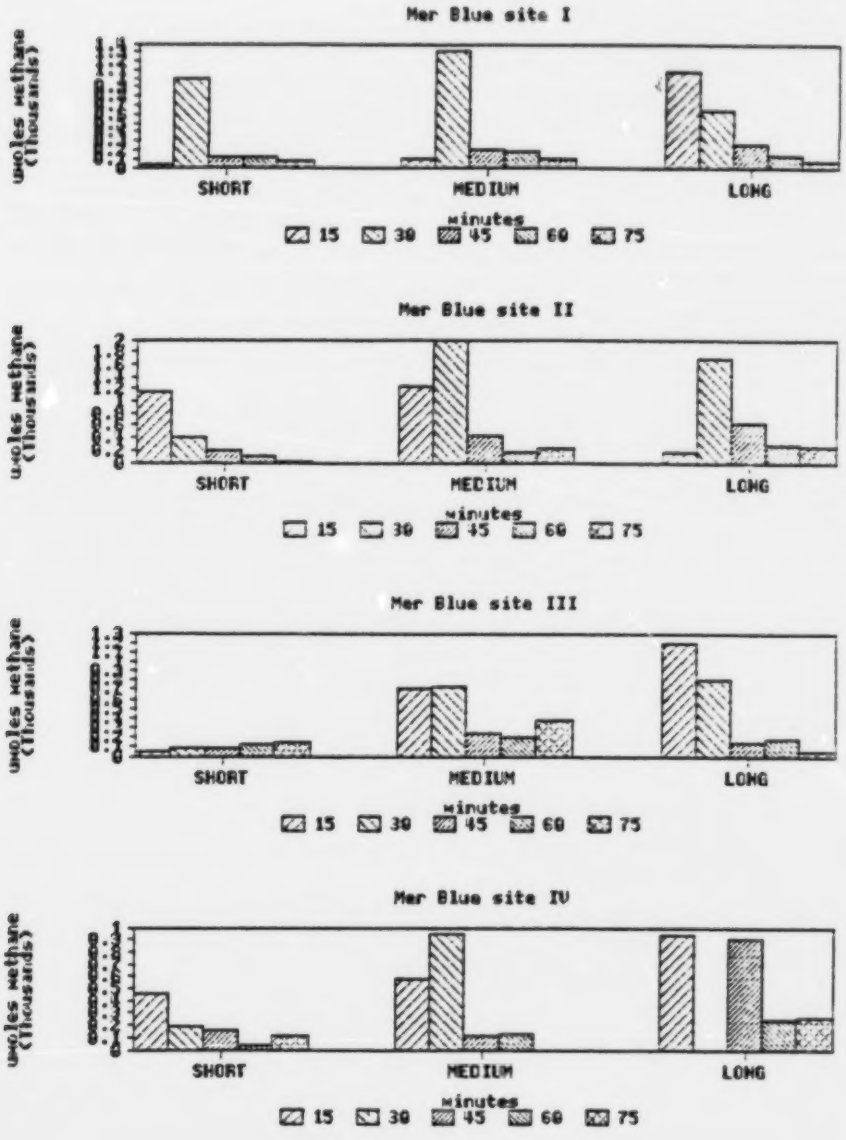
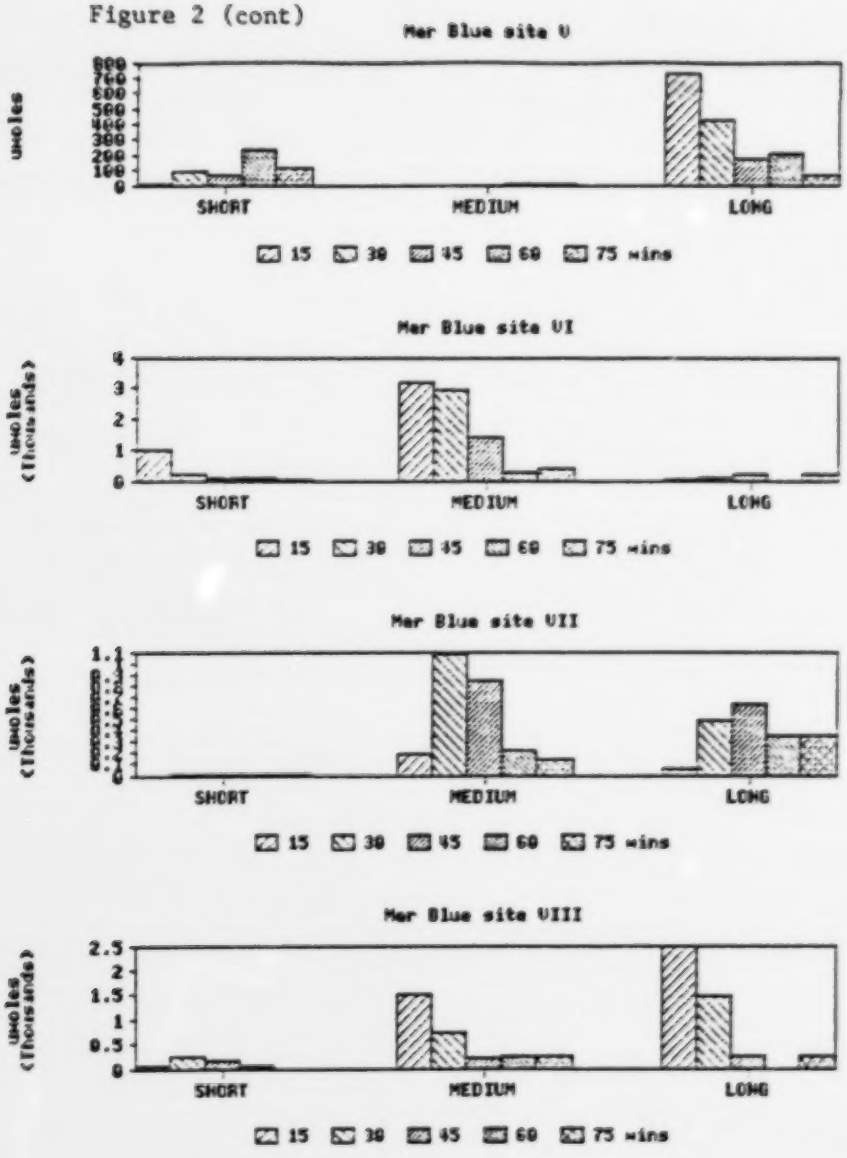

Mar Blue site I
umoles methane (thousands)
SHORT
MEDIUM
LONG
minutes
15 30 45 60 75
Mar Blue site II
umoles methane (thousands)
SHORT
MEDIUM
LONG
minutes
15 30 45 60 75
Mar Blue site III
umoles methane (thousands)
SHORT
MEDIUM
LONG
minutes
15 30 45 60 75
Mar Blue site IV
umoles methane (thousands)
SHORT
MEDIUM
LONG
minutes
15 30 45 60 75

Figure 2 (cont)



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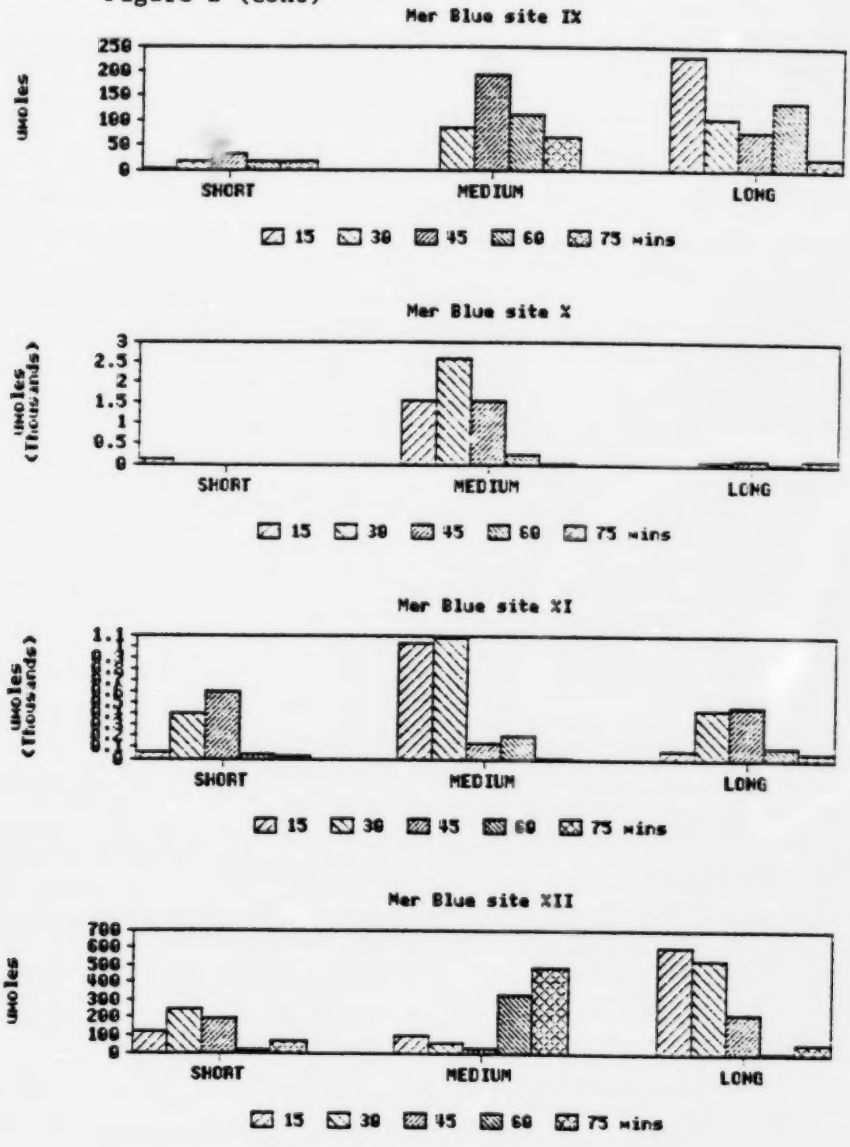

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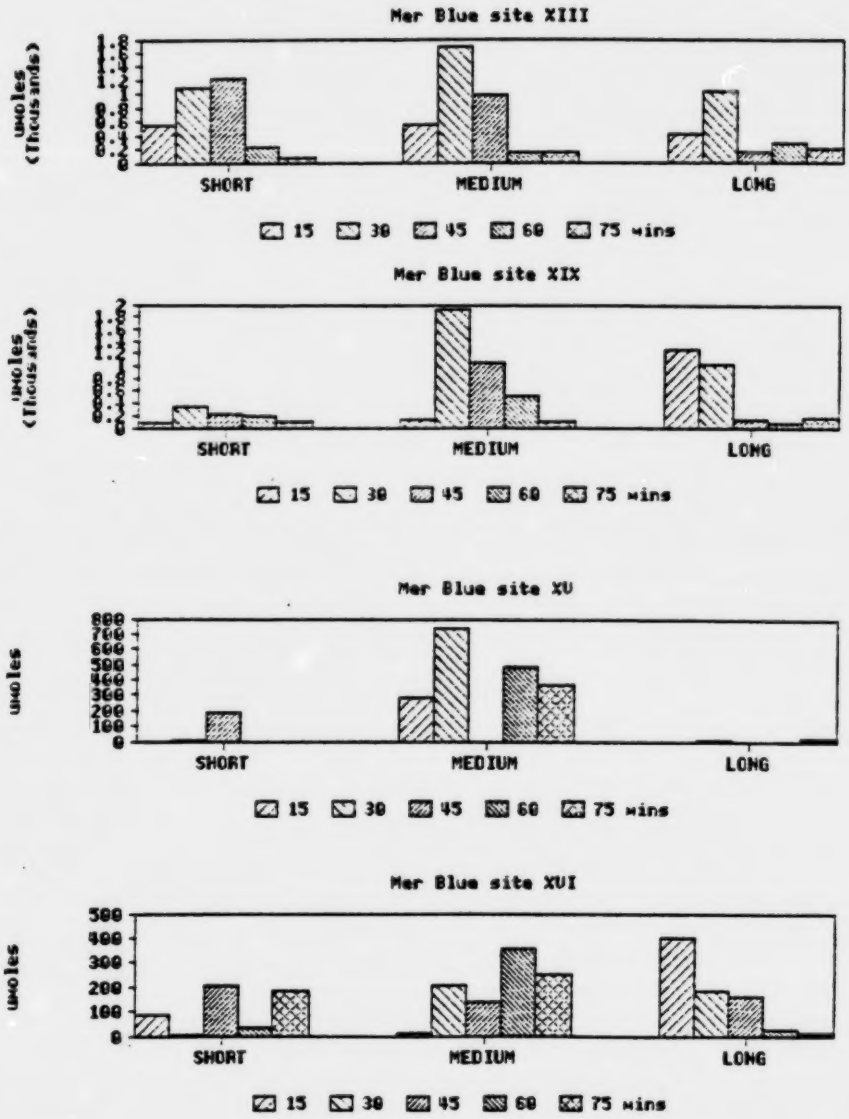

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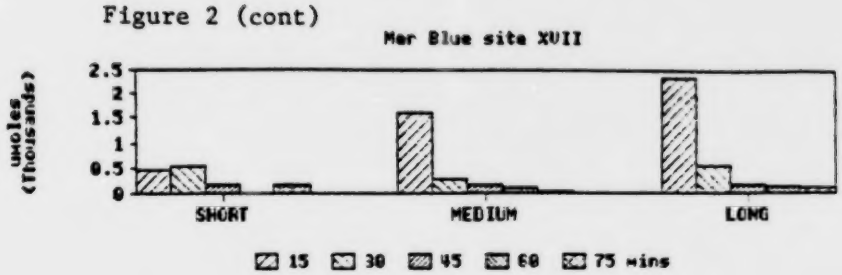

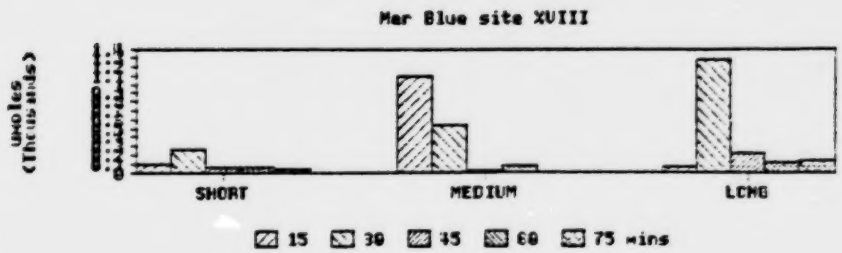

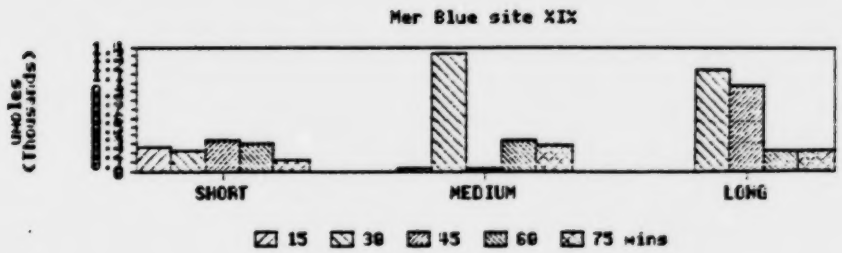

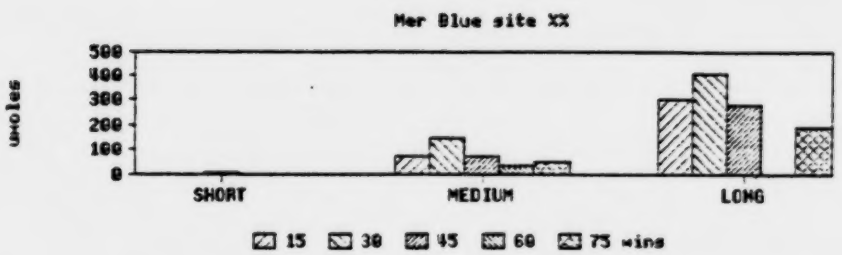

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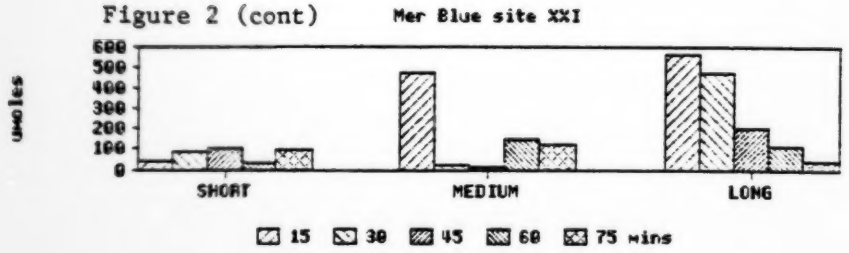
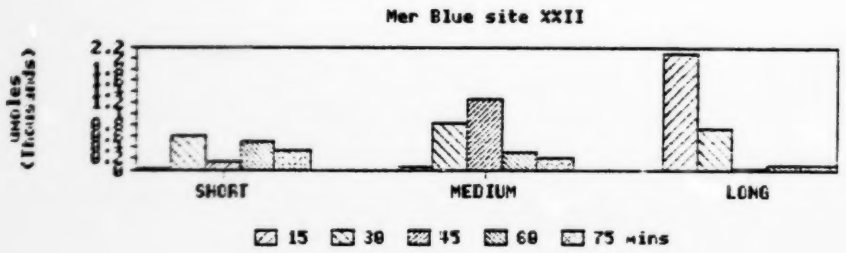
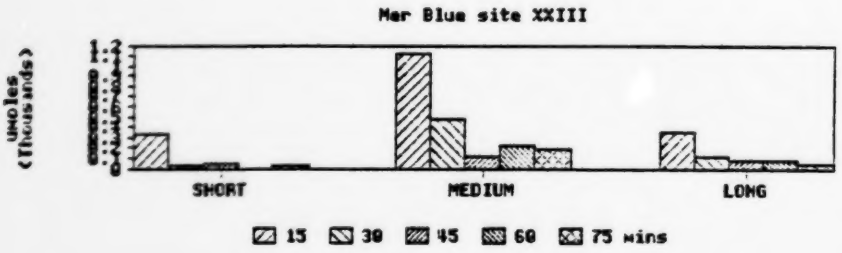
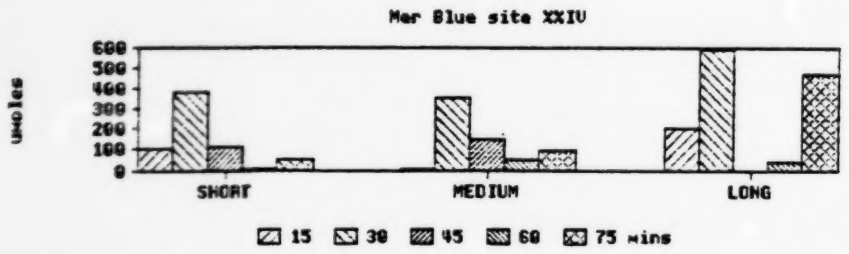








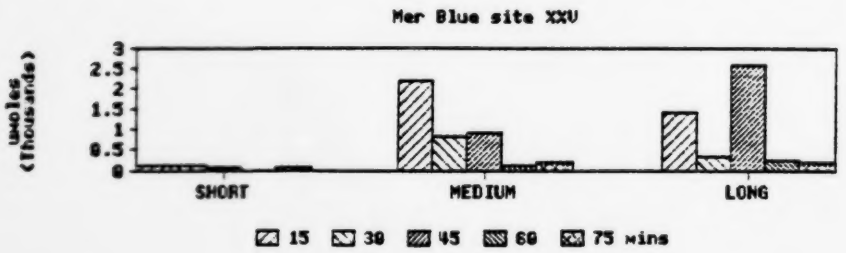


Figure 3

METHANE in mmoles

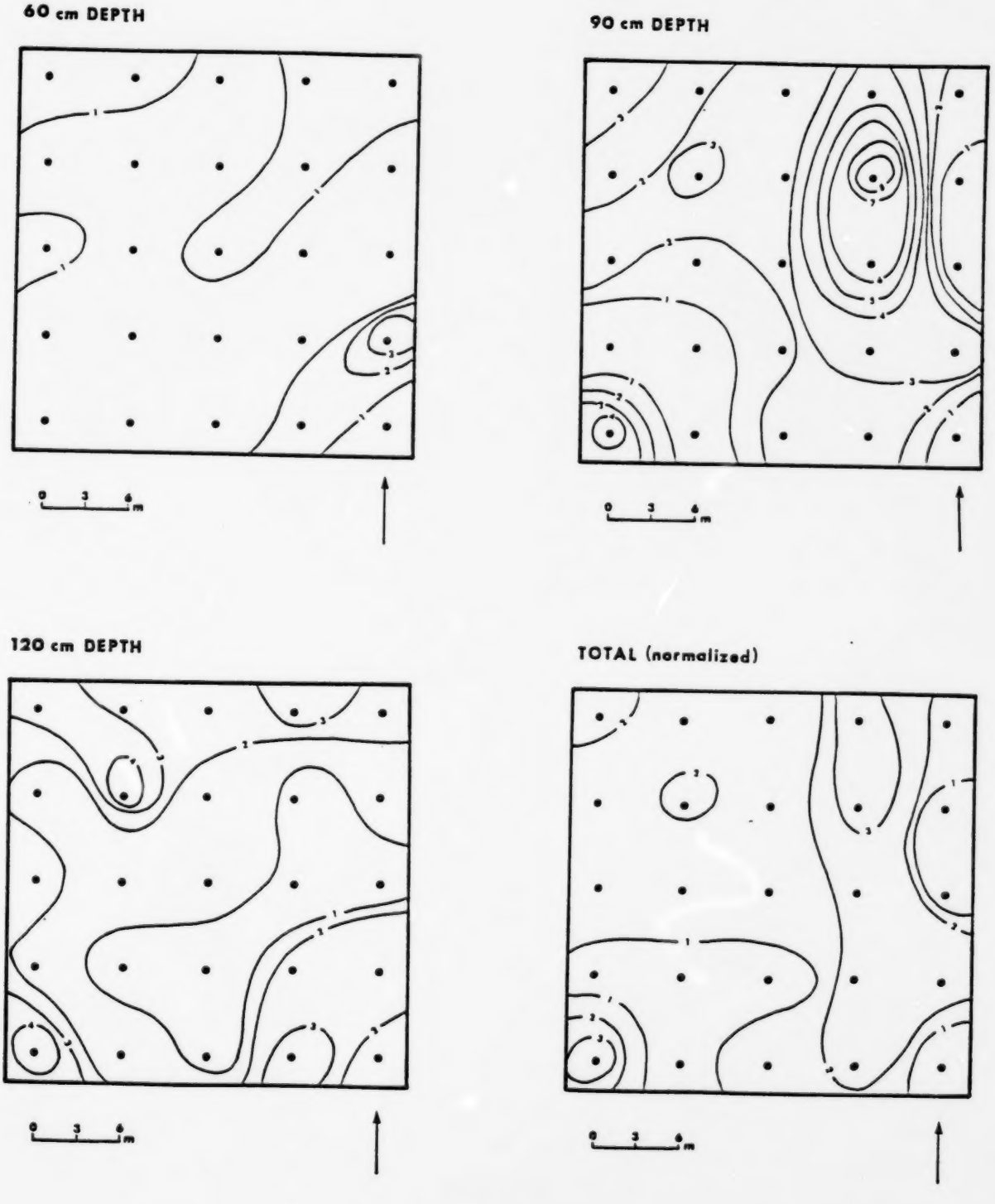
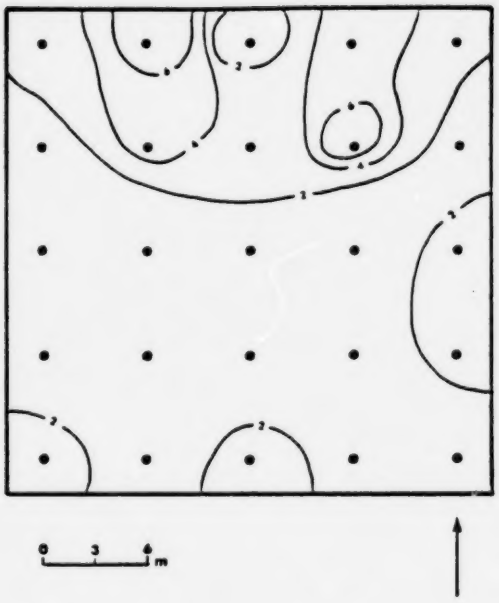


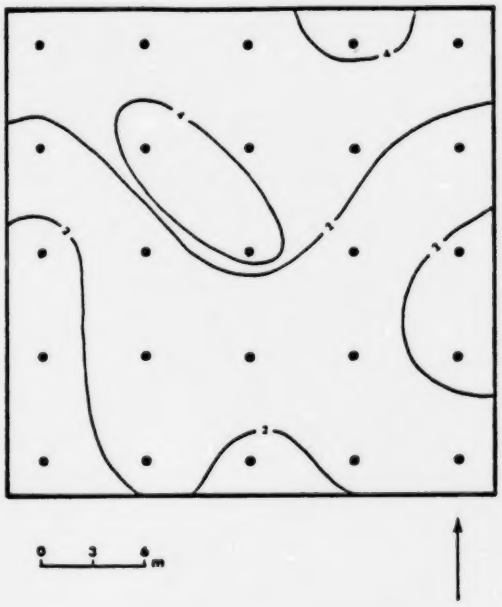
Figure 4

WATER in cl

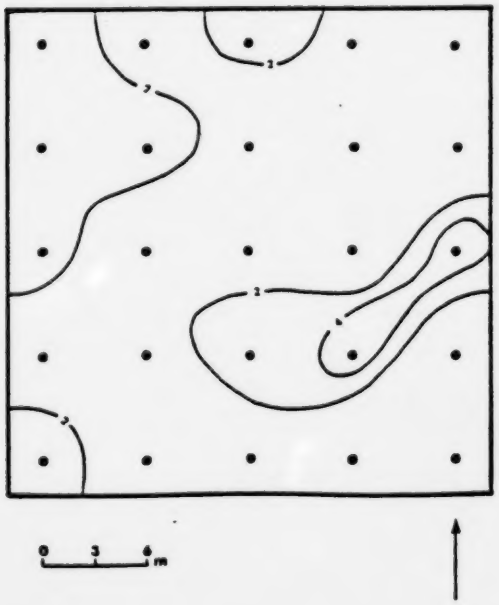
60 cm DEPTH



90 cm DEPTH



120 cm DEPTH



TOTAL (normalized)

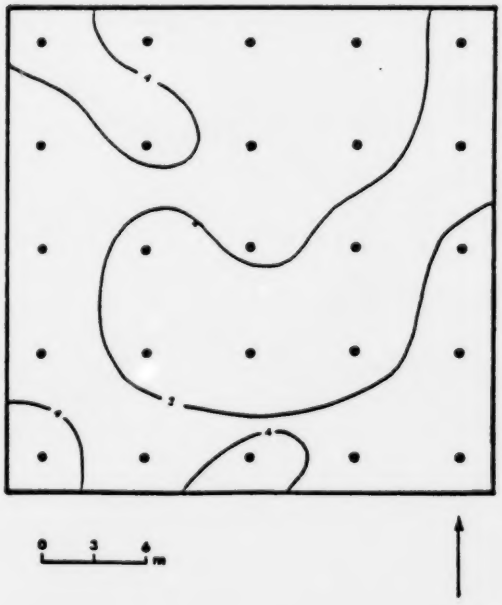
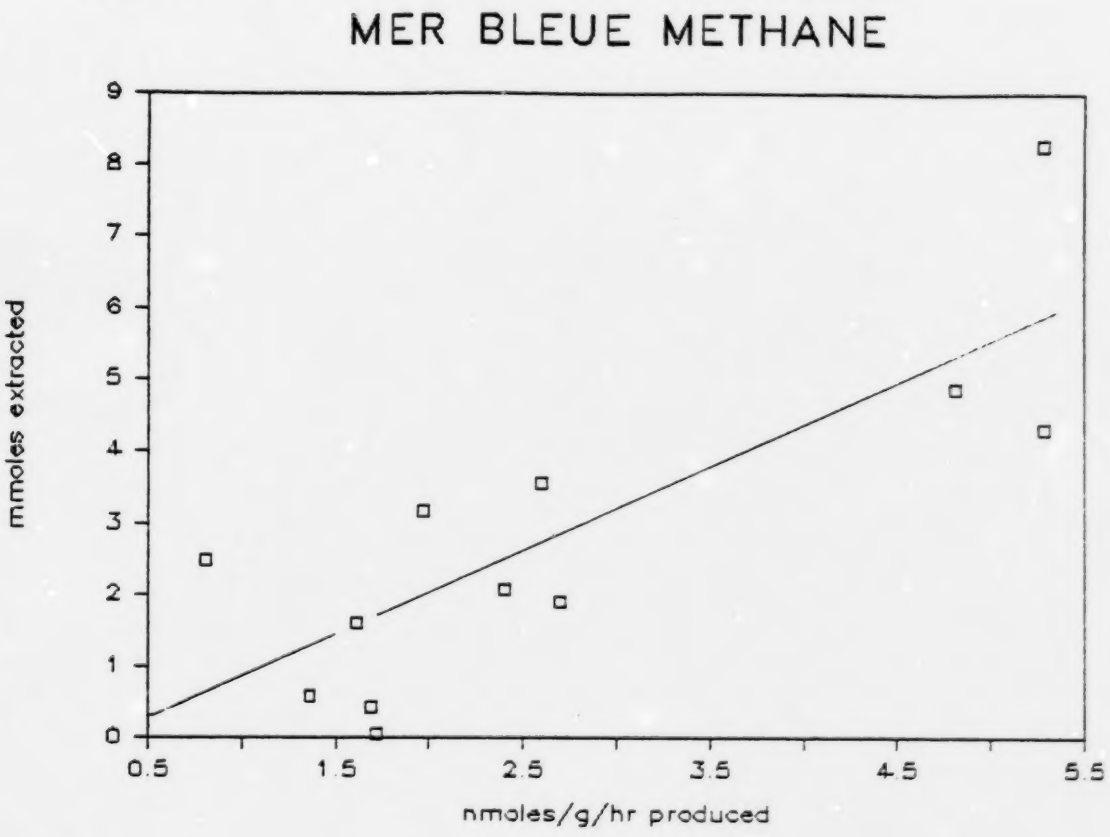


Figure 5


MER BLEUE METHANE
nmoles extracted
0
1
2
3
4
5
6
7
8
9
0.5
1.5
2.5
3.5
4.5
5.5
nmoles/g/hr produced

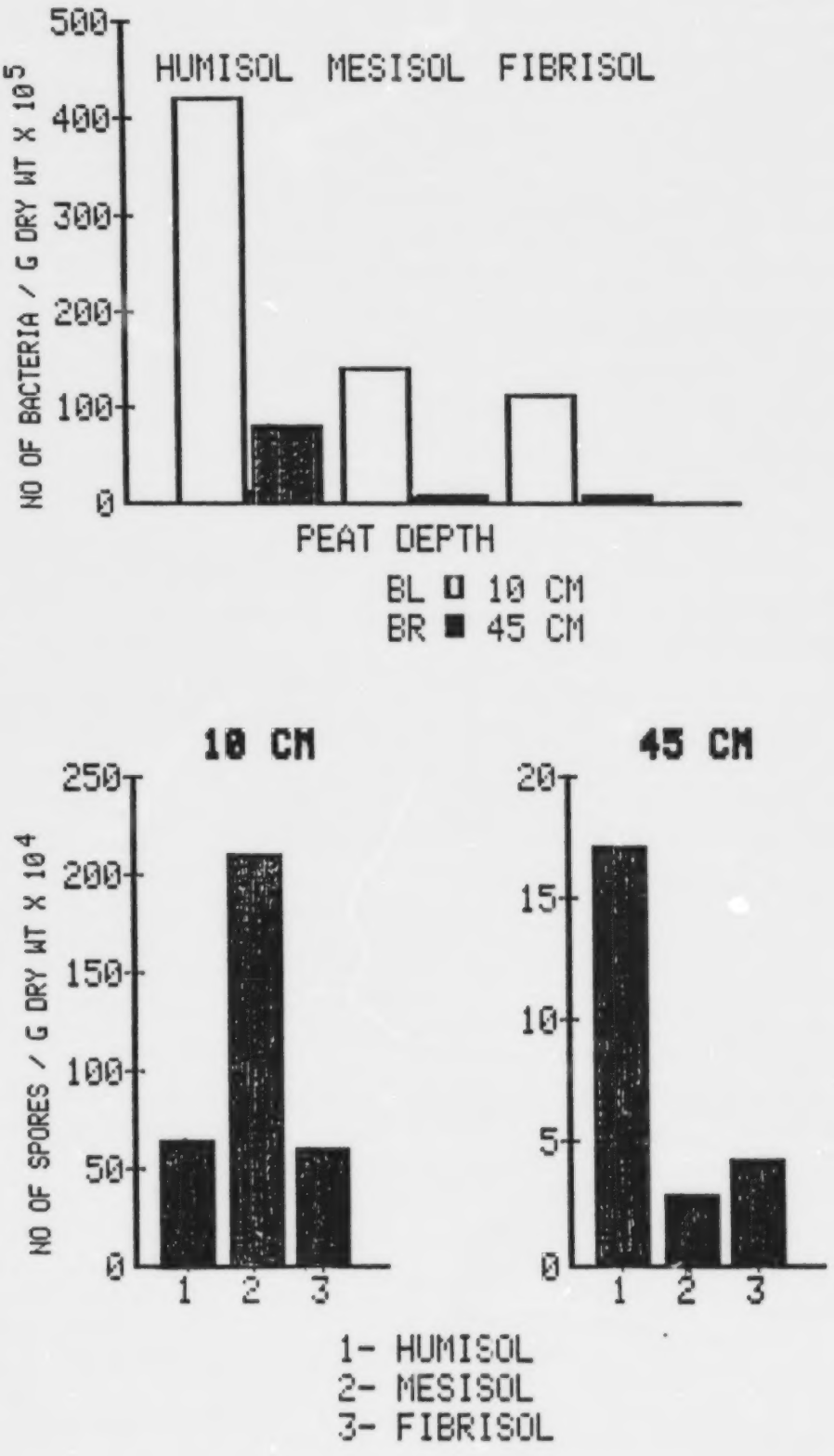


Figure 6. Viable counts of bacteria and bacterial spores at two depths (10 and 45 cm) in three types of muskeg bogs (humisol, mesisol, fibrisol).

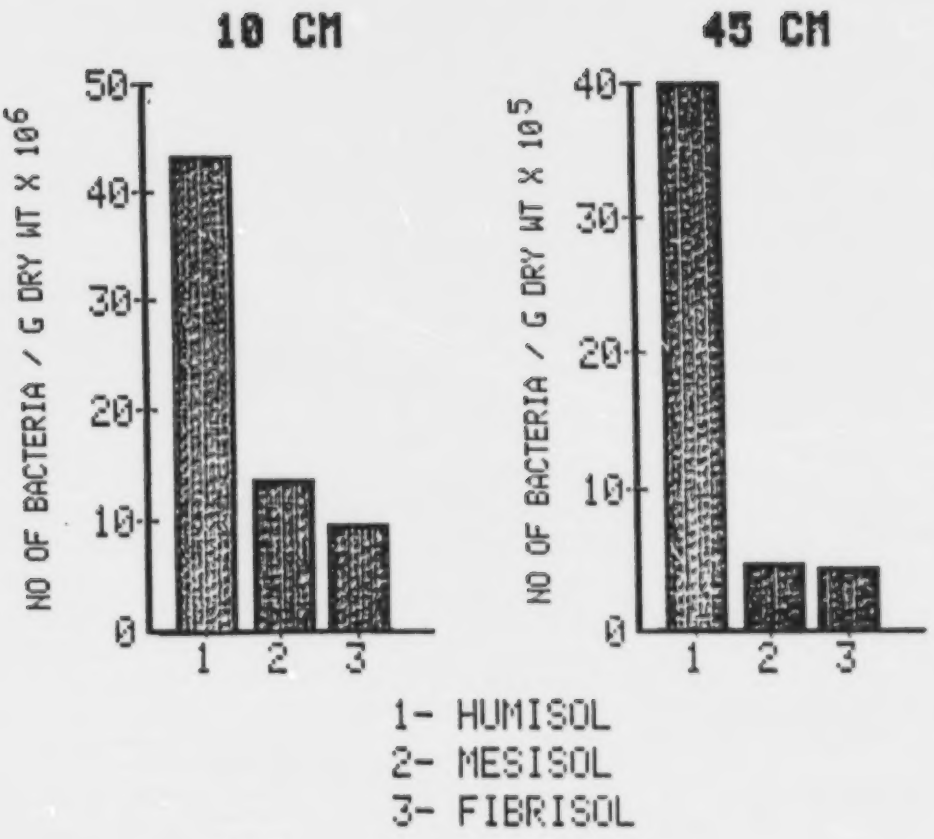


Figure 7. Aerobic cellulolytic bacteria at two depths (10 and 45 cm) in three different muskeg bogs.

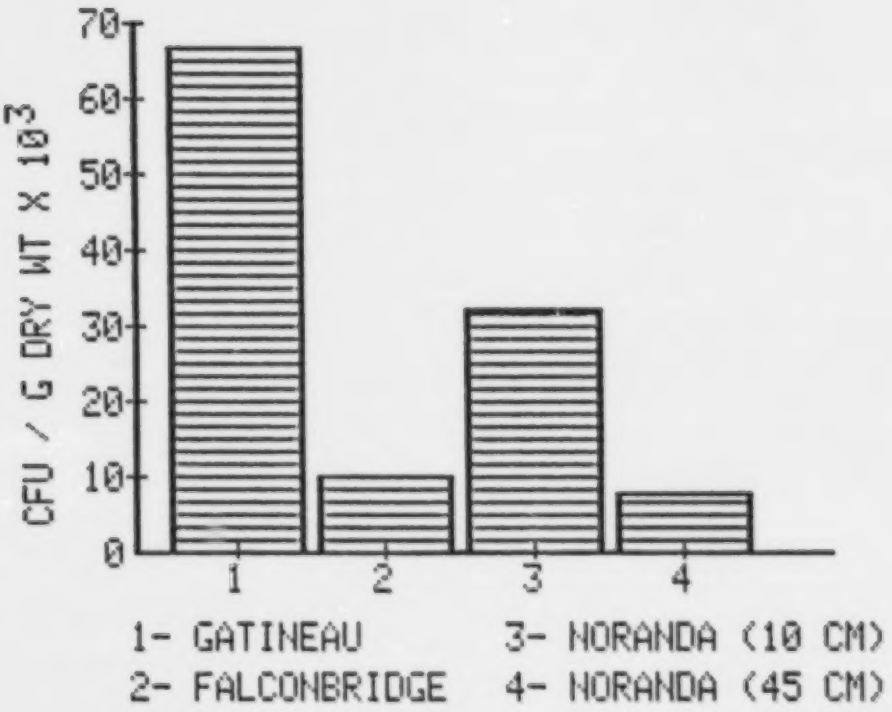


Figure 8. Fungi, in colony forming units in surface samples and in deeper layers of peat.